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UTILITY PATENT APPLICATION TRANSMITTAL
(Large Entity)*(Only for new nonprovisional applications under 37 CFR 1.53(b))*Docket No.
DEX-0054

Total Pages in this Submission

TO THE ASSISTANT COMMISSIONER FOR PATENTSBox Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

**NOVEL MUTATIONS IN HUMAN MLH1 AND HUMAN MSH2 GENES USEFUL IN DIAGNOSING
COLORECTAL CANCER**

and invented by:

Robbins et al.If a **CONTINUATION APPLICATION**, check appropriate box and supply the requisite information:☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: _____

Which is a:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: _____

Which is a:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: _____

Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 30 pages and including the following:
 - a. ☒ Descriptive Title of the Invention
 - b. ☒ Cross References to Related Applications *(if applicable)*
 - c. ☐ Statement Regarding Federally-sponsored Research/Development *(if applicable)*
 - d. ☐ Reference to Microfiche Appendix *(if applicable)*
 - e. ☒ Background of the Invention
 - f. ☒ Brief Summary of the Invention
 - g. ☐ Brief Description of the Drawings *(if drawings filed)*
 - h. ☒ Detailed Description
 - i. ☒ Claim(s) as Classified Below
 - j. ☒ Abstract of the Disclosure

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UTILITY PATENT APPLICATION TRANSMITTAL (Large Entity)

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Total Pages in this Submission

Application Elements (Continued)

3. ☐ Drawing(s) (when necessary as prescribed by 35 USC 113)
- a. ☐ Formal Number of Sheets _____
- b. ☐ Informal Number of Sheets _____
4. ☒ Oath or Declaration
- a. ☐ Newly executed (original or copy) ☒ Unexecuted
- b. ☐ Copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional application only)
- c. ☒ With Power of Attorney ☐ Without Power of Attorney
- d. ☐ DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s) named in the prior application,
see 37 C.F.R. 1.63(d)(2) and 1.33(b).
5. ☐ Incorporation By Reference (usable if Box 4b is checked)
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. ☐ Computer Program in Microfiche (Appendix)
7. ☒ Nucleotide and/or Amino Acid Sequence Submission (if applicable, all must be included)
- a. ☒ Paper Copy
- b. ☒ Computer Readable Copy (identical to computer copy)
- c. ☒ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers (cover sheet & document(s))
9. ☐ 37 CFR 3.73(B) Statement (when there is an assignee)
10. ☐ English Translation Document (if applicable)
11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations
12. ☐ Preliminary Amendment
13. ☒ Acknowledgment postcard
14. ☒ Certificate of Mailing

☐ First Class ☒ Express Mail (Specify Label No.): EL429958222US

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Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed)

16. ☐ Additional Enclosures (please identify below):

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	8	- 20 =	0	x \$18.00	\$0.00
Indep. Claims	5	- 3 =	2	x \$78.00	\$156.00
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>					\$0.00
BASIC FEE					\$760.00
OTHER FEE (specify purpose) _____					\$0.00
TOTAL FILING FEE					\$916.00

- ☒ A check in the amount of \$916.00 to cover the filing fee is enclosed.
- ☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. 12-1086 as described below. A duplicate copy of this sheet is enclosed.
- ☐ Charge the amount of _____ as filing fee.
- ☒ Credit any overpayment.
- ☒ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Jane Massey Licata

Signature

Dated: October 22, 1999

Jane Massey Licata
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DEX-0054

CERTIFICATE OF EXPRESS MAILING

"Express Mail" Label No.EL429958222US

Date of Deposit OCTOBER 22, 1999

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the "BOX SEQUENCE", Assistant Commissioner for Patents, Washington, D.C. 20231.

- 1) Patent Application Transmittal Letter (2 copies);
- 2) Application consisting of 30 pages of Specification, including two (2) pages of Claims, and one (1) page of Abstract;
- 3) Unexecuted Declaration and Power of Attorney.
- 4) Statement to Support Filing and Submission in Accordance with 37 CRF §§1.821-1.825;
- 5) Sequence Listing;
- 6) Diskette containing computer readable copy of Sequence Listing;
- 7) Return Post Card; and
- 8) Check in the amount of \$916.00.

Jane Massey Licata
JANE MASSEY LICATA

NOVEL MUTATIONS IN HUMAN MLH1 AND HUMAN MSH2 GENES USEFUL
IN DIAGNOSING COLORECTAL CANCER

INTRODUCTION

This application claims the benefit of U.S. provisional
5 application Serial No. 60/105,180, filed October 22, 1998.

BACKGROUND OF THE INVENTION

Colorectal cancer (CRC) is one of the most common fatal
cancers in developed countries, and the worldwide incidence
is increasing. The United States and the United Kingdom are
10 high incidence countries, with an estimated 133,500 new cases
and 55,300 deaths (Parker et al. CA Cancer J. Clin. 1996 46:5-
27) in the United States and 30,941 cases and approximately
17,000 deaths in the United Kingdom (HMSO UK Cancer Registry
Data). The population lifetime risk is 1 in 25 in the United
15 States and Northern Europe and thus represents a significant
public health issue (Sharp et al. Cancer Registration
Statistics Scotland 1981-1990, Information and Statistics
Division, The National Health Service in Scotland, Edinburgh
(1993)). Identification of people who are predisposed to the
20 disease would allow targeting of effective preventative
measures with the aim of reducing the considerable cancer
related mortality (Burke et al. J. Am. Med. Ass'n. 1997
227:915-919).

One group of people with a very high colorectal cancer
25 risk are those who carry germline mutations in genes that
participate in DNA mismatch repair. hMSH2 (Fishel et al. Cell
1993 75:1027-1038; Leach et al. Cell 1993 75:1215-1225; U.S.
Patent 5,591,826) and hMLH1 (Bronner et al. Nature 1994
368:258-261; Papadopoulos et al. Science 1994 263:1625-1629;

PCT Publication No. WO 95/20678, published on August 3, 1995) are the two genes most commonly involved in heredity predisposition to CRC, but mutations in hPMS1 and hPMS2 also occur in a minority of cases (Nicolaidis et al. Nature 1994 5 371:75-80). Such mutations are usually associated with marked familial aggregation of colorectal, uterine and other cancers constituting the clinically defined autosomal dominant syndrome of hereditary non-polyposis colorectal cancer (HNPCC) (Lynch et al. Gastroenterology 1993 104:1535-1549; Liu et al. 10 Nature Med. 1996 2:169-174; Wijnen et al. Am. J. Hum. Genet. 1995 56:1060-1066; Mary et al. Hum. Mol. Genet. 1994 3:2067-2069; Nystrom-Lahti et al. Nature Med. 1995 1:1203-1206). However, an appreciable proportion of patients who have early onset colorectal cancer but who do not fulfill pragmatic 15 criteria for HNPCC (Vasen et al. Dis. Colon Rectum 1991 34:424-425) also carry mismatch repair gene mutations (Liu et al. Nature Med. 1995 2:169-174; Dunlop et al. Br. Med. J. 1997 314:1779-1780). Thus, restricting genetic testing to individuals from families fulfilling HNPCC criteria is likely 20 to exclude a significant fraction of gene carriers in the general population. However, screening unselected patients with sporadic cancer represents an enormous workload and may provide a very low yield of mutation carriers (Liu et al. Nat. Med. 1995 1:348-352; Tomlinson et al. J. Med. Genet. 1997 25 34:39-42).

It is clear that issues concerning indications for genetic testing and interpretation of results are critical in hereditary cancer syndromes (Giardiello et al. N. Engl. J. Med. 1997 336: 823-827).

30 Using a population-based approach, factors indicative of the likelihood of identifying patients with mismatch repair gene mutations were investigated. Improved approaches to mutation detection and the prevalence of detectable mismatch repair gene alterations in various screened groups who were

not selected on the basis of family history were also determined.

SUMMARY OF THE INVENTION

An object of the present invention is to provide novel,
5 variant hMLH1 sequences.

Another object of the present invention is to provide novel, variant hMSH2 sequences.

Another object of the present invention is to provide a method of diagnosing hereditary non-polyposis colorectal
10 cancer in a patient or determining a patient's susceptibility to developing hereditary non-polyposis colorectal cancer via detection of novel variant hMLH1 or hMSH2 sequences or the exonic or intronic sequences of the hMLH1 and hMSH2 genes.

Another object of this invention is to provide methods
15 and compositions for identifying new variants of hMLH1 and hMSH2 genes.

Yet another object of the present invention is to provide experimental models of hereditary non-polyposis colorectal cancer.

20 DETAILED DESCRIPTION OF THE INVENTION

To better elucidate the structure of human MLH1 and human MSH2 genes and to determine possible sites of alternative splicing, the genes were cloned and sequenced and PCR was used to determine alternate splice products (variants)
25 and exon/intron boundaries. Elucidation of intron/exon boundary sequences revealed that hMLH1 is encoded by 19 coding exons. The hMLH1 gene sequence was determined by PCR.

The intron/exon structure of the hMLH1 is shown below. Positions of introns that interrupt the hMLH1 cDNA are shown.
30 Exonic sequence is presented in upper case and intronic sequence in lower case letters. Exons are numbered from the 5' end of the cDNA sequence.

hMLH1 Exon 1

aggcactgaggtgattggc (SEQ ID NO:1)
tgaaggcacttccgttgagcatctagacgtttccttggctcttctggcgccaaa (SEQ ID NO:2)
5 ATGTCGTTCTGTGGCAGGGGTTATTCGGCGGCTGGACGAGACAGTGGTGAACCGCATCGC
GGCGGGGAAGTTATCCAGCGGCCAGCTAATGCTATCAAAGAGATGATTGAGAACTG (SEQ ID NO:3)
gtacggagggagtcgagccgg (SEQ ID NO:4)
gctcacttaagggctacga (SEQ ID NO:5)
10 cttaacgg (SEQ ID NO:6)

hMLH1 Exon 2

aatatgtacattagagtagttg (SEQ ID NO:7)
cagactgataaattattttctgtttgatttgccag (SEQ ID NO:8)
TTTAGATGCAAAATCCACAAGTATTCAAGTGATTGTTAAAGAGGGAGGCCTGAAGTTGA
15 TTCAGATCCAAGACAATGGCACCGGGATCAGG (SEQ ID NO:9)
gtaagtaaaacctcaaagtagcaggatgtttgtgcgcttcatggaa (SEQ ID NO:10)
gagtcaggacctttctctg (SEQ ID NO:11)

hMLH1 Exon 3

agagatttggaaaatgagtaac (SEQ ID NO:12)
20 atgattatttactcatcttttttggtatctaacag (SEQ ID NO:13)
AAAGAAGATCTGGATATTGTATGTGAAAGGTTCACTACTAGTAAACTGCAGTC
CTTTGAGGATTTAGCCAGTATTTCTACCTATGGCTTTCGAGGTGAG (SEQ ID NO:14)
gtaagctaaagattcaagaaatgtgtaaaatat (SEQ ID NO:15)
cctcctgtgatgacattgt (SEQ ID NO:16)
25 c

hMLH1 Exon 4

aacctttcccttttggtgagg (SEQ ID NO:17)
tgacagtgggtgaccagcagtgagtttttctttcagtctattttcttttcttccttag
(SEQ ID NO:18)
30 GCTTTGGCCAGCATAAGCCATGTGGCTCATGTTACTATTACAACGAAAACAGCT
GATGGAAAGTGTGCATACAG (SEQ ID NO:19)

gtatagtgtgctgacttcttttactcatatatattcattctgaaatgtattttgg (SEQ ID NO:20)

gcctaggtctcagagtaatc (SEQ ID NO:21)

hMLH1 Exon 5

5 ttgatat (SEQ ID NO: 22)

gattttctcttttccccttggg (SEQ ID NO:23)

attagtatctatctctctactggatattaatttgttatattttctcattag (SEQ ID NO: 24)

AGCAAGTTACTCAGATGGAAAAGTAAAGCCCCTCCTAAACCATGTGCTGGCAATCAAG

10 GGACCCAGATCACG (SEQ ID NO: 25)

gtaagaatggtacatgggaca (SEQ ID NO:26)

gtaaattgttgaagctttgtttg (SEQ ID NO:27)

hMLH1 Exon 6

gggtttttattttcaagtacttctatg (SEQ ID NO: 28)

15 aatttacaagaaaaatcaatcttctgttcag (SEQ ID NO: 29)

GTGGAGGACCTTTTTTACAACATAGCCACGAGGAGAAAAGCTTTAAAAAATCCAAGT

GAAGAATATGGGAAAATTTTGGAAGTTGTTGGCAG (SEQ ID NO:30)

gtacagtcctaaaatctgggagtggtgtctctgagatttgtcatcaaagtaatgtgttctagt
(SEQ ID NO:31)

20 gctcatacattgaacagttgctgagc (SEQ ID NO:32)

hMLH1 Exon 7

ctagtgtgtgtttttggc (SEQ ID NO:33)

aactcttttcttactcttttggttttcttttccag (SEQ ID NO:34)

GTATTCAGTACACAATGCAGGCATTAGTTTCTCAGTTAAAAAA (SEQ ID NO:35)

25 gtaagttcttggtttatgggggatggttttggttttatgaaaagaaaaaaggggattttt

aatagtttgct (SEQ ID NO:36)

ggtggagataagggttatg (SEQ ID NO:37)

hMLH1 Exon 8

ctcagccatgagacaataaatcc (SEQ ID NO:38)

30 ttgtgtcttctgctgtttgttttatcag (SEQ ID NO:39)

CAAGGAGAGACAGTAGCTGATGTTAGGACACTACCCAATGCCTCAACCGTGGACAATAT
TCGCTCCATCTTTGGAAATGCTGTTAGTCG (SEQ ID NO:40)

gtatgtcgataacctatatataaaaaaatcttttacatttattatcttggtttatcatt (SEQ
ID NO:41)

5 ccatcacattatttgggaacc (SEQ ID NO: 42)

hMLH1 Exon 9

caaaagcttcagaatctc (SEQ ID NO: 43)

ttttctaataag (SEQ ID NO:44)

AGAACTGATAGAAATTGGATGTGAGGATAAAACCCTAGCCTTCAAAATGAATGGTTACA
10 TATCCAATGCAAACACTACTCAGTGAAGAAGTGCATCTTCTTACTCTTCATCAACC (SEQ
ID NO:45)

gtaagttaaaaagaaccacatgggaaat (SEQ ID NO:46)

ccactcacaggaaacacccacag (SEQ ID NO:47)

hMLH1 Exon 10

15 catgacttttgtgtgaatgtacacc (SEQ ID NO: 48)

tgtgacctcaccctcaggacagttttgaactggttgctttctttttattgttttag (SEQ
ID NO:49)

ATCGTCTGGTAGAATCAACTTCCTTGAGAAAAGCCATAGAAACAGTGTATGCAGCCTATT
TGCCCAAAAACACACACCCATTCTGTACCTCAG (SEQ ID NO:50)

20 gtaatgtagcaccaaactcctcaaccaagactcacaaggaa (SEQ ID NO:51)

cagatgttctatcaggctctcctc (SEQ ID NO: 52)

hMLH1 Exon 11

gggctttttctccccctccc (SEQ ID NO:53)

actatctaaggtaattgttctctcttattttcctgacag (SEQ ID NO: 54)

25 TTTAGAAATCAGTCCCCAGAATGTGGATGTTAATGTGCACCCCACAAAGCATGAAG
TTCCTTCTGACGAGGAGAGCATCCTGGAGCGGGTGCAGCAGCACATCGAGAGCAAG
CTCCTGGGCTCCAATTCCTCCAGGATGTACTTCACCCAG (SEQ ID NO:55)

gtcagggcgcttctcatccagctacttctctggggcctttgaaatgtgcccgccaga
(SEQ ID NO:56)

30 cgtgagagcccagatttt (SEQ ID NO:57)

hMLH1 Exon 12aattatacctcatactagc (SEQ ID NO:58)ttcttttcttagtactgctccatttggggacctgtatatctatacttcttattctgagtct
ctccactatatatatatatatatatattttttttttttttttttttttaatacag (SEQ

5 ID NO:59)

ACTTTGCTACCAGGACTTGCTGGCCCCCTCTGGGGAGATGGTTAAATCCACAACAAGTCT
GACCTCGTCTTCTACTTCTGGAAGTAGTGATAAGGTCTATGCCCACCAGATGGTTCGTA
CAGATTCCCGGGAACAGAAGCTTGATGCATTTCTGCAGCCTCTGAGCAAACCCC
TGTCCAGTCAGCCCCAGGCCATTGTCACAGAGGATAAGACAGATATTTCTAGTGGCAGGG
10 CTAGGCAGCAAGATGAGGAGATGCTTGAACCTCCAGCCCCCTGCTGAAGTGGCTGCCAAAA
ATCAGAGCTTGGAGGGGGGATACAACAAAGGGGACTTCAGAAATGTCAGAGAAGAGAGGAC
CTACTTCCAGCAACCCCCAG (SEQ ID NO:60)

gtatggccttttgggaaaagtacagccta (SEQ ID NO:61)

cctcctttattctgtaataaaac (SEQ ID NO:62)15 **hMLH1 Exon 13**tgcaaccacaaaaatttggc (SEQ ID NO:63)taagtttaaaaaacaagaataataatgatctgcacttccttttcttcattgcag (SEQ ID
NO:64)

AAAGAGACATCGGGAAGATTCTGATGTGGAAATGGTGGGAAGATGATTCCCGAAAGGAAA

20 TGA CTGCAGCTTGTACCCCCCGGAGAAGGATCATTAACTCACTAGTGTTTTGAGTCTCCAG
GAAGAAATTAATGAGCAGGGACATGAGG (SEQ ID NO:65)gtacgtaaacgctgtggcctgcctgggatgcatagggcctcaactgcaa (SEQ ID NO:
66)ggttttggaaatggagaaag (SEQ ID NO:67)25 **hMLH1 Exon 14**tggtgtctctagttctgg (SEQ ID NO: 68)tgcctgggtgctttgggtcaatgaagtgggggttggtaggattctattacttacctgttttt
tggtttttatttttttgttttgcag (SEQ ID NO:69)

TTCTCCGGGAGATGTTGCATAACCACTCCTTCGTGGGCTGTGTGAATCCTCAGTGGGCCTTG

30 GCACAGCATCAAACCAAGTTATACCTTCTCAACACCACCAAGCTTAG (SEQ ID NO:70)

gtaaatacagctgagtgtgtgaacaa (SEQ ID NO:71)

gcagagctactacaacaatg (SEQ ID NO: 72)

hMLH1 Exon 15

cccatttgtcccaactgg SEQ ID NO:73

ttgtatctcaagcatgaattcagcttttccttaaagtcacttcatttttattttcag (SEQ ID NO:74)

5 TGAAGAACTGTTCTACCAGATACTCATTTATGATTTTGCCAATTTTGGTGTTCTCAGGTTATCG (SEQ ID NO:75)

gtaagtttagatccttttcactt (SEQ ID NO:76)

ctgacatttcaactgaccg (SEQ ID NO:77)

hMLH1 Exon 16

10 catttgggatgctccgttaaagc (SEQ ID NO:78)

ttgctccttcatgttcttgcttcttcctag (SEQ ID NO:79)

GAGCCAGCACCGCTCTTTGACCTTGCCATGCTTGCCCTTAGATAGTCCAGAGAGTGGCTG
GACAGAGGAAGATGGTCCCAAAGAAGGACTTGCTGAATACATTGTTGAGTTTCTGAAGA
AGAAGGCTGAGATGCTTGACAGACTATTTCTCTTTGGAAATTGATGAG (SEQ ID NO:80)

15 gtgtgacagccattcttatacttctgttgattctc (SEQ ID NO:81)

caaataaaatttccagccgggtg (SEQ ID NO:82)

hMLH1 Exon 17

ggaaaggcactggagaaatggg (SEQ ID NO:83)

at ttgttttaactatgacagcattatttcttgttcccttgctcctttttcctgcaagcag

20 (SEQ ID NO:84)

GAAGGGAACCTGATTGGATTACCCCTTCTGATTGACAACTATGTGCCCCCTTTGGAGGG
ACTGCCTATCTTCATTCTTCGACTAGCCACTGAG (SEQ ID NO:85)

gtcagtgatcaagcagataactaagcattt (SEQ ID NO:86)

cggtacatgcatgtgtgctggaggg (SEQ ID NO:87)

25 **hMLH1 Exon 18**

taagtagtctgtgatctccg (SEQ ID NO:88)

tttagaatgagaatgtttaaattcgtacctattttgaggtattgaatttctttggaccag
(SEQ ID NO:89)

GTGAATTGGGACGAAGAAAAGGAATGTTTTGAAAGCCTCAGTAAAGAATGCGCTATGTT

30 CTATTCCATCCGGAAGCAGTACATATCTGAGGAGTCGACCCTCTCAGGCCAGCAG (SEQ ID NO:90)

gtacagtggatgatgcacactggcaccacaggacta (SEQ ID NO:91)

ggacaggacctcatacat (SEQ ID NO:92)

hMLH1 Exon 19

gacaccagtgtatgttgg (SEQ ID NO:93)

5 gatgcaaacagggaggcttatgacatctaattgtgttttccag (SEQ ID NO:94)

AGTGAAGTGCCTGGCTCCATTCCAAACTCCTGGAAGTGGACTGTGGAACACATTGTC

TATAAAGCCTTGCGCTCACACATTCTGCCTCCTAAACATTTACAGAAGATGGAAATATC

CTGCAGCTTGCTAACCTGCCTGATCTATACAAAGTCTTTGAGAGGTGTTAA (SEQ ID NO:95)

10 atatggttatttatgcactgt (SEQ ID NO:96)

gggatgtgttcttcttctc (SEQ ID NO:97)

tgtattccgatacaaagtgttgtatcaaagtgtgatatacaaagtgtaccaacataagtg
(SEQ ID NO:98)

Elucidation of intron/exon boundary sequences revealed
15 that hMSH2 is encoded by 16 coding exons. The hMSH2 gene
sequence was determined by PCR.

The intron/exon structure of the hMSH2 is shown below.
Positions of introns that interrupt the hMSH2 cDNA are shown.
Exonic sequence is presented in upper case and intronic
20 sequence in lower case letters. Exons are numbered from the
5' end of the cDNA sequence.

hMSH2 Exon 1

ggcgggaaacagcttagtgggtgtgggggtcg (SEQ ID NO:99)

cgcattttcttcaaccagga (SEQ ID NO:100)

25 ggtgaggagggttttcgac (SEQ ID NO:101)

ATGGCGGTGCAGCCGAAGGAGACGCTGCAGTTGGAGAGCGCGGCCGAGGTCGGCTTCGTG

CGCTTCTTTTCAGGGCATGCCGAGAGAAGCCGACCACACAGTGCGCCTTTTCGACCGGGG

CGACTTCTATACGGCGCACGGCGAGGACGCGCTGCTGGCCGCCCGGGAGGTGTTCAAGA

CCCAGGGGGTGATCAAGTACATGGGGCCGGCAG (SEQ ID NO:102)

30 gtgagggccgggac (SEQ ID NO:103)

ggcgcggtgctggggagg (SEQ ID NO:104)

gac

hMSH2 Exon 2

gaa

gtccagctaatacacagtgccttg (SEQ ID NO:105)

aacatgtaatatctcaaatctgtaatgtactttttttttttttaag (SEQ ID NO:106)

5 GAGCAAAGAATCTGCAGAGTGTGTGCTTAGTAAAATGAATTTTGAATCTTTTGTA
GATCTTCTTCTGGTTCGTCAGTATAGAGTTGAAGTTTATAAGAATAGAGCTGGAAATAAG
GCATCCAAGGAGAATGATTGGTATTTGGCATATAAG (SEQ ID NO:107)

gtaattatcttccttttttaatttacttattttt (SEQ ID NO:108)

ttaagagtagaaaaataaaaaatgtg (SEQ ID NO:109)

10 aag

hMSH2 Exon 3

ATTAATAAGGtTCATAGAGTTTGGATTTTTCCTtTTTtgc (SEQ ID NO:110)

ttataaaaatttttaaagtatgttcaag (SEQ ID NO:111)

agtttggttaaattttttaaatttttatttttacttag (SEQ ID NO:112)

15 GCTTCTCCTGGCAATCTCTCTCAGTTTGAAGACATTCTCTTTGGTAACAATGAT
ATGTCAGCTTCCATTGGTGTGTGGGTGTTAAAATGTCCGCAGTTGATGGCCAGAGACAG
GTTGGAGTTGGGTATGTGGATTCCATACAGAGGAACTAGGACTGTGTGAATTCCTGAT
AATGATCAGTTCTCCAATCTTGAGGCTCTCCTCATCCAGATTGGACCAAAGGAATGTGT
TTTACCCGGAGGAGAGACTGCTGGAGACATGGGGAACTGAGACAG (SEQ ID NO:113)

20 gtaagcaaattgagtcctagtgat (SEQ ID NO:114)

agaggagattccaggcctaggaaag (SEQ ID NO:115)

gc

TCTTTAATTGACATGATACTG (SEQ ID NO:116)

hMSH2 Exon 4

25 ttca

tttttgctttttcttattccttttc (SEQ ID NO:117)

tcatagtagtttaaactatttcttttcaaaatag (SEQ ID NO:118)

ATAATTCAAAGAGGAGGAATTCTGATCACAGAAAGAAAAAAGCTGACTTTTCCACAAA
AGACATTTATCAGGACCTCAACCGGTTGTTGAAAGGCAGGAGAGCAGATGAATA

30 GTGCTGTATTGCCAGAAATGGAGAATCAG (SEQ ID NO:119)

gtacatggattataaatgtgaattacaatatataatgtaaataatgtaatatataata
aataatatgtaaactatagtgacttt (SEQ ID NO:120)
ttagaaggatatttctgtca (SEQ ID NO:121)
tat

5 hMSH2 Exon 5

actggcacca (SEQ ID NO:122)
gtggtatagaaatcttcgattttt (SEQ ID NO:123)
aaattcttaatttttag (SEQ ID NO:124)
GTTGCAGTTTCATCACTGTCTGCGGTAATCAAGTTTTTTAGAACTCTTATCAGATGATTC
10 CAACTTTGGACAGTTTGAAGTACTACTTTTGACTTCAGCCAGTATATGAAATTGGATA
TTGCAGCAGTCAGAGCCCTTAACCTTTTTTCAG (SEQ ID NO:125)
gtaaaaaaaaaaaaaaaaaaaaa (SEQ ID NO:126)
aaaagggttaaaaaatgttgatt (SEQ ID NO:127)
gg
15 TTAAAAAATGTTT (SEQ ID NO:128)
t
caTTGACATATACTGAAGAAGCT (SEQ ID NO:129)
TATAAAGGAGCTAAAATATTTGGAAAT (SEQ ID NO:130)
att
20 ATTATACTTGGATTAGATAACTAGCTTTAAATGGGTGTATTTT (SEQ ID NO:131)

hMSH2 Exon 6

gtt
ttcactaatgagcttgccattc (SEQ ID NO:132)
tttctattttattttttgtttactag (SEQ ID NO:133)
25 GGTTCTGTTGAAGATAACCACTGGCTCTCAGTCTCTGGCTGCCTTGCTGAATAAGTGTA
AACCCCTCAAGGACAAAGACTTGTTAACCAGTGGATTAAGCAGCCTCTCATGGATAAGA
ACAGAATAGAGGAGAG (SEQ ID NO:134)
gtatgttattagtttataactttcgttagttttatgtaacctgca (SEQ ID NO:135)
gttaccacatgattatacc (SEQ ID NO:136)
30 ac

hMSH2 Exon 7

ga

cttacgtgcttagttgataa (SEQ ID NO:137)

attttaattttataactaaaatatattttacattaattcaagttaattttatttcag (SEQ ID
5 NO:138)

ATTGAATTTAGTGGAAGCTTTTGTAGAAGATGCAGAATTGAGGCAGACTTTACAAGAAG
ATTTACTTCGTCGATTCCCAGATCTTAACCGACTTGCCAAGAAGTTTCAAAGACAAGCA
GCAAACCTTACAAGATTGTTACCGACTCTATCAGGGTATAAATCAACTACCTAATGTTAT
ACAGGCTCTGGAAAAACATGAAG (SEQ ID NO:139)

10 gtaacaagtgatttttggtttttttg (SEQ ID NO:140)

ttttccttcaactcatacaatata (SEQ ID NO:141)

tac

hMSH2 Exon 8

ga

15 tttgtattctgtaaaatgagatcttt (SEQ ID NO:142)

ttatttggttggttttactacttttcttttag (SEQ ID NO:143)

GAAAACACCAGAAATTATTGTTGGCAGTTTTTGTGACTCCTCTTACTGATCTTCGTTCT
GACTTCTCCAAGTTTCAGGAAATGATAGAAACAACCTTTAGATATGGATCAG (SEQ ID
NO:144)

20 gtatgcaatatacttttttaatttaag (SEQ ID NO:145)

cagtagttattttttaaaaagcaaag (SEQ ID NO:146)

gcc

hMSH2 Exon 9

gt

25 ctttacccattatttataggatt (SEQ ID NO:147)

ttgtcacttttggttctggttgag (SEQ ID NO:148)

GTGGAAAACCATGAATTCCTTGTAACCTTCATTTGATCCTAATCTCAGTGAA
TTAAGAGAAATAATGAATGACTTGGAAGAAGATGCAGTCAACATTAATAAGTGCAGC
CAGAGATCTTG (SEQ ID NO:149)

30 gtaagaatgggtcattggag (SEQ ID NO:150)

gttgggaataattcttttgctat (SEQ ID NO:151)

ac

hMSH2 Exon 10

gg

tagtaggtatatttatggaataactttt (SEQ ID NO:152)

tcttttcttcttgtttatcaag (SEQ ID NO:153)

5 GCTTGGACCCTGGCAAACAGATTAAACTGGATTCCAGTGCACAGTTTGGATATTACTTTC

GTGTAACCTGTAAGGAAGAAAAAGTCCTTCGTAACAATAAAAACTTTAGTACTGTAGATA

TCCAGAAGAATGGTGTAAATTTACCAACAG (SEQ ID NO:154)

gtttgtaagtcattattatatttttaaccctttatt (SEQ ID NO:155)

aattccctaaatgctctaaca (SEQ ID NO:156)

10 tg

hMSH2 Exon 11

ca

cattgcttctagtacacattt (SEQ ID NO:157)

taatatttttaataaaaactgttatttcgatttgcag (SEQ ID NO:158)

15 CAAATTGACTTCTTTAAATGAAGAGTATACCAAATAAAACAGAATATGAAGAAGCCC

AGGATGCCATTGTAAAGAAATTGTCAATATTTCTTCAG (SEQ ID NO:159)

gtaaacttaatagaactaa (SEQ ID NO:160)

taatgttctgaatgtcacctg (SEQ ID NO:161)

g

20 hMSH2 Exon 12

at

tcagtattcctgtgtacattt (SEQ ID NO:162)

tctgttttttattttttatacag (SEQ ID NO:163)

GCTATGTAGAACCAATGCAGACACTCAATGATGTGTAGCTCAGCTAGATGCTGTTGTC

25 AGCTTTGCTCACGTGTCAAATGGAGCACCTGTTCCATATGTACGACCAGCCATTTTGGAGAA

AGGACAAGGAAGAATTATATTAAGCATCCAGGCATGCTTGTGTTGAAGTTCAAGATG

AAATTGCATTTATTCCTAATGACGTATACTTTGAAAAAGATAAACAGATGTTCCACATC
ATTACTG (SEQ ID NO:164)

gtaaaaaacctggttt (SEQ ID NO:165)

30 ttgggctttgtgggggtaa (SEQ ID NO:166)

cg

hMSH2 Exon 13

cg

cgattaatcatcagtgtac (SEQ ID NO:167)

agtttaggactaacaatccatttattagtagcagaaagaagtttaaaatcttgctttct

5 gatataatttggtttgtag (SEQ ID NO:168)

GCCCCAATATGGGAGGTAAATCAACATATATTCGACAAACTGGGGTGATAGTACT

CATGGCCCAAATTGGGTGTTTTGTGCCATGTGAGTCAGCAGAAGTGTCCATTGTGGACTG

CATCTTAGCCCGAGTAGGGGCTGGTGACAGTCAATTGAAAGGAGTCTCCACGTTTCATGGC

TGAAATGTTGGAAACTGCTTCTATCCTCAG (SEQ ID NO:169)

10 gtaagtgcacatctcctagtcctt (SEQ ID NO:170)

gaagatagaaatgtatgtctctg (SEQ ID NO:171)

tcc

hMSH2 Exon 14

ta

15 ccacattttatgtgatgggaa (SEQ ID NO:172)

atttcatgtaattatgtgcttcag (SEQ ID NO:173)

GTCTGCAACCAAAGATTCATTAATAATCATAGATGAATTGGGAAGAGGAACTTCTACCTA

CGATGGATTTGGGTTAGCATGGGCTATATCAGAATACATTGCAACAAAGATTGGTGCTTT

TTGCATGTTTGCAACCCATTTTCATGAACTTACTGCCTTGGCCAATCAGATACCAACTGT

20 TAATAATCTACATGTCACAGCACTCACCCTGAAGAGACCTTAACCTATGCTTTATCAGGT

GAAGAAAG (SEQ ID NO:174)

gtatgtactattggagtactctaaattcagaacttg

gtaatgggaaacttactacc (SEQ ID NO:175)

cc

25 hMSH2 Exon 15

ct

cttctcatgctgtcccctc (SEQ ID NO:176)

acgcttccccaaatttcttatag (SEQ ID NO:177)

GTGTCTGTGATCAAAGTTTTGGGATTCATGTTGCAGAGCTTGCTAATTTCCCTAAGCAT

30 GTAATAGAGTGTGCTAAACAGAAAGCCCTGGAAGTTGAGGAGTTTCAGTATATTGGAGA

ATCGCAAGGATATGATATCATGGAACCAGCAGCAAAGAAGTGCTATCTGGAAAGAGAG

(SEQ ID NO:178)

gtttgtcagtttggtttt (SEQ ID NO:179)

catagttttaacttagcttctc (SEQ ID NO:180)

tat

hMSH2 Exon 16

ta

5 attactcatgggacattcaca (SEQ ID NO:181)

tgtgttttcag (SEQ ID NO:182)

CAAGGTGAAAAAATTATTCAGGAGTTCCTGTCCAAGGTGAAACAAATGCCCTTTAC

TGAAATGTCAGAAGAAAACATCACATAAAGTTAAAACAGCTAAAAGCTGAAGTAATAGC

AAAGAATAATAGCTTTGTAAATGAAATCATTTACGAATAAAAGTTACTACGTGA (SEQ
10 ID NO:183)

aaa

atcccagtaatggaatgaag (SEQ ID NO:184)

gta

hMLH1 and hMSH2 genes were sequenced in 50 cancer
15 patients (age of onset <30) and 26 random anonymous donors.
Initial genomic sequencing detected 12 germline mutations in
12 patients (24%). Five mutations were found in hMLH1, and
7 in hMSH2. Using a combination of genomic sequencing and *in*
vitro synthesized-protein-truncation assay (IVSP), a total of
20 15 germ-line mutations were identified. The mutations are
described in Table 1.

**Table 1: Pathogenic hMLH1 and hMSH2 Mutations Identified in
Young Colorectal Cancer Probands**

Gene and Patient	Mutation	Nucleotide Change	Effect on Coding Sequence	Location
hMLH1:				
329	616delAAG	Deletion of AAG at 1846- 1848	Deletion of Lys616	Exon 16
533	IVS8- 3delTA	Deletion of TA at 677-3	Splice mutation	IVS 8

5	696	K618A	AA→GC at 1852-1853	Lys→Ala at 618	Exon 16
	804	R659X	C→T at 1975	Arg→Stop at 659	Exon 17
	815	IVS1+1G→A	G→A at 116+1	Splice mutation	IVS 1
	817	del exon 13	Deletion of ~3 kb involving IVS 12 through exon 13 to IVS 13	Deletion of codons 470-520 (exon 13)	IVS 12-13, exon 13
	889		not identified	Truncation of IVSP	Exons 12-19
10	hMSH2:				
	528	R406X	C→T at 1216	Arg→Stop at 406	Exon 7
	579	H639Y IVS13-1G→T	C→T at 1915 G→T at 2211	Double mutation results in deletion of codons 588-820 (exons 12-14)	Exon 12, IVS 13
	814	Q601X	C→T at 1801	Gln→Stop at 601	Exon 12
	818	Q252X	C→T at 754	Gln→Stop at 252	Exon 4
	825	delCTGT	Deletion of CTGT at 808-811	Deletion of codons 265-314 (exon 5)	Exon 5
	830	R680X	C→T at 2038	Arg→Stop at 680	Exon 13
	1157	M1L	A→T at 1	New initiation at codon 26	Exon 1

"IVS" means intervening sequence.

15 Two of the mutations identified in Table 1 for hMLH1 and three of the mutations identified in Table 1 for hMSH2 are believed

to be new. For hMLH1, these include: the splice mutation IVS1 + 1G→A in patient 815, also referred to herein as "hMLH1 mutant 1"; and deletion of exon 13 in patient 817, also referred to herein as "hMLH1 mutant 2". For hMSH2, these include the double mutation H639Y IVS13-1G→T leading to deletion of codons 588-820 in patient 579, also referred to herein as "hMSH2 mutant 1", mutation R680X in patient 830 which comprises a nucleotide change from C to T at position 2038 in Exon 13 and results in a stop codon at position 680 of the coding sequence, also referred to herein as "hMSH2 mutant 2"; and mutation M1L in patient 1157 which comprises a nucleotide change from A to T at position 1 resulting in a new initiation at codon 26, also referred to herein as "hMSH2 mutant 3". Detection of these genetic mutations is useful in diagnosing HNPCC in a patient and determining susceptibility of a patient for developing HNPCC.

There are several methodologies available from recombinant DNA technology which may be used for detecting these new variants and identifying additional genetic mutations responsible for colon cancer. The identification of intronic sequences of hMLH1 and hMSH2 provided herein is particularly useful for design of intronic such as those exemplified in SEQ ID NO:1, 5, 7, 11, 12, 16, 17, 21, 23, 27, 28, 32, 33, 37, 38, 42, 43, 47, 48, 52, 53, 57, 58, 62, 63, 67, 68, 72, 73, 77, 78, 82, 83, 87, 88, 92, 93, 97, 100, 104, 105, 109, 111, 115, 117, 121, 123, 121, 123, 127, 129, 132, 136, 137, 141, 142, 146, 147, 151, 152, 156, 157, 161, 162, 166, 167, 171, 172, 175, 176, 180, 181 and 184 for use in identifying mutants in the splice donor or acceptor sites of the hMLH1 or hMSH2 gene. Examples of methodologies useful in detecting and identifying new variants of these genes include, but are not limited to, direct probing, ligase chain reaction (LCR) and polymerase chain reaction (PCR) methodology.

Detection of variants or mutants using direct probing involves the use of oligonucleotide probes which may be

prepared synthetically or by nick translation. In a preferred embodiment, the probes are complementary to at least a portion of the variant hMLH1 or hMSH2 genes identified herein. The DNA probes may be suitably labeled using, for example, a radiolabel, enzyme label, fluorescent label, or biotin-avidin label, for subsequent visualization in for example a Southern blot hybridization procedure. The labeled probe is reacted with a sample of DNA from a patients suspected of having HNPCC bound to nitrocellulose or Nylon 66 substrate. The areas that carry DNA sequences complementary to the labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling may then be visualized, for example, by autoradiography.

Alternative probe techniques, such as ligase chain reaction (LCR) involve the use of a mismatch probe, i.e., probes which have full complementarity with the target except at the point of the mutation or variation. The target sequence is then allowed to hybridize both with the oligonucleotides having full complementarity, i.e., oligonucleotides complementary to the hMLH1 or hMSH2 variants of the present invention, and oligonucleotides containing a mismatch under conditions which will distinguish between the two. By manipulating the reaction conditions, it is possible to obtain hybridization only where there is full complementarity. If a mismatch is present, then there is significantly reduced hybridization.

The polymerase chain reaction (PCR) is a technique that amplifies specific DNA sequences. Repeated cycles of denaturation, primer annealing and extension carried out with a heat stable enzyme Taq polymerase leads to exponential increases in the concentration of desired DNA sequences.

Given the knowledge of nucleotide sequences encoding the hMLH1 and hMSH2 genes, it is possible to prepare synthetic oligonucleotides complementary to the sequences which flank the DNA of interest. Each oligonucleotide is complementary

to one of the two strands. The DNA is then denatured at high temperatures (e.g., 95°C) and then reannealed in the presence of a large molar excess of oligonucleotides. The oligonucleotides, oriented with their 3' ends pointing towards
5 each other, hybridize to opposite strands of the target sequence and prime enzymatic extension along the nucleic acid template in the presence of the four deoxyribonucleotide triphosphates. The end product is then denatured again for another cycle. After this three-step cycle has been repeated
10 several times, amplification of a DNA segment by more than one million fold can be achieved. The resulting DNA may then be directly sequenced in order to locate any genetic alterations. Alternatively, the identified hMLH1 and hMSH2 variants of the present invention make it possible to prepare oligonucleotides
15 that will only bind to altered DNA, so that PCR will only result in the multiplication of the DNA if the mutation is present. Following PCR, allele-specific oligonucleotide hybridization may be used to detect the colon cancer point mutation.

20 Alternatively, an adaptation of PCR called amplification of specific alleles (PASA) can be employed; this method uses differential amplification for rapid and reliable distinction between alleles that differ at a single base pair. Newton et al. Nucleic Acid Res. 1989 17:2503; Nichols et al. Genomics
25 1989 5:535; Okayama et al. J. Lab. Clin. Med. 1989 1214:105; Sarkar et al. Anal. Biochem. 1990 186:64; Sommer et al. Mayo Clin. Proc. 1989 64:1361; Wu, Proc. Nat'l Acad. Sci. USA 1989 86:2757; and Dutton et al. Biotechniques 1991 11:700. PASA involves amplification with two oligonucleotide primers such
30 that one is allele specific. The desired allele is efficiently amplified, while the other allele(s) is poorly amplified because it mismatches with a base at or near the 3' end of the allele specific primer. Thus, PASA or the related method PAMSA can be used to specifically amplify one or more
35 mutant hMLH1 or hMSH2 alleles. Where such amplification is

performed on genetic material obtained from a patient, it can serve as a method of detecting the presence of one or more mutant hMLH1 and/or hMSH2 alleles in a patient. PCR-induced mutation restriction analysis, often referred to as IMRA, can
5 also be used in the detection of mutants.

Also important is the development of experimental models of HNPCC. Such models can be used to screen for agents that alter the degenerative course of HNPCC. Having identified specific mutations in the hMLH1 and hMSH2 genes as a cause of
10 HNPCC, it is possible using genetic manipulation, to develop transgenic model systems and/or whole cell systems containing a mutated hMLH1 and/or hMSH2 gene or a portion thereof. The model systems can be used for screening drugs and evaluating the efficacy of drugs in treating HNPCC. In addition, these
15 model systems provide a tool for defining the underlying biochemistry of hMLH1 and hMSH2 and their relationship to HNPCC, thereby providing a basis for rational drug design.

One type of cell system which can be used in the present invention can be naturally derived. For this, blood samples
20 from an affected individual are obtained and permanently transformed into a lymphoblastoid cell line using, for example, Epstein-Barr virus. Once established, such cell lines can be grown continuously in suspension cultures and can be used in a variety of *in vitro* experiments to study hMLH1
25 and hMSH2 expression and processing. Another cell line used in these studies comprises skin fibroblasts derived from patients.

The mutated gene can also be excised for use in the creation of transgenic animals containing the mutated gene.
30 For example, the hMLH1 and hMSH2 variants of the present invention can each be cloned and placed in a cloning vector. Examples of cloning vectors which can be used include, but are not limited to, lCharon35, cosmid, or yeast artificial chromosome. The variant hMLH1 or hMSH2 gene can then be
35 transferred to a host nonhuman knockout animal such as a

knockout mouse. As a result of the transfer, the resultant transgenic nonhuman animal will preferably express one or more of the variant hMLH1 or hMSH2 polypeptides.

Alternatively, minigenes encoding variant hMLH1 or hMSH2 polypeptides can be designed. Such minigenes may contain a cDNA sequence encoding a variant hMLH1 or hMSH2 polypeptide, preferably full-length, a combination of hMLH1 or hMSH2 exons, or a combination thereof, linked to a downstream polyadenylation signal sequence and an upstream promoter (and preferably enhancer). Such a minigene construct will, when introduced into an appropriate transgenic host, such as a mouse or rat, express a variant hMLH1 or hMSH2 polypeptide.

One approach to creating transgenic animals is to target a mutation to the desired gene by homologous recombination in an embryonic stem (ES) cell *in vitro* followed by microinjection of the modified ES cell line into a host blastocyst and subsequent incubation in a foster mother. Frohman et al. Cell 1989 56:145. Alternatively, the technique of microinjection of the mutated gene, or portion thereof, into a one-cell embryo followed by incubation in a foster mother can be used. Additional methods for producing transgenic animals are well known in the art.

Transgenic animals are used in the assessment of new therapeutic compositions and in carcinogenicity testing, as exemplified by U.S. Patent 5,223,610. These animals are also used in the development of predictive animal models for human disease states, as exemplified in U.S. Patent 5,221,778. Therefore, the novel mutations of the hMLH1 and hMSH2 genes of the present invention, which are believed to cause HNPCC, provide a useful means for developing knockout transgenic animals to assess this disease.

Site directed mutagenesis and/or gene conversion can also be used to mutate a non human hMLH1 or hMSH2 gene allele, either endogenously or via transfection, such that the

mutated gene encodes a polypeptide with an altered amino acid as described in the present invention.

In addition, antibodies to the hMLH1 or hMSH2 gene and variants thereof can be raised for use in the examination of the function of the truncated transcripts of the hMLH1 or hMSH2 gene. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the hMLH1 and hMSH2 genes of the present invention can be obtained by direct injection into an animal or by administering the gene to an animal, preferably a nonhuman. The antibody so obtained will then bind the hMLH1 or hMSH2 gene or itself. In this manner, even a fragment of the gene can be used to generate these antibodies.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler et al. Nature 1975 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al. Immunology Today 1983 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al. in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., 1985, pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to the hMLH1 or hMSH2 genes of this invention. Also, transgenic mice may be used to express humanized antibodies to the hMLH1 or hMSH2 genes of this invention.

The following nonlimiting examples are provided to further illustrate the present invention.

EXAMPLES**Example 1: Patients and Samples**

A total of 76 subjects were studied: 50 unrelated patients diagnosed with colorectal cancer at <30 years of age and 26 anonymous donors. There were 15 male and 11 female anonymous donors who were cancer free at the time of sampling and whose mean ages was 41 years. None of the study subjects were referred specifically because of a family history of colon cancer. All cancer patients had histologically confirmed colorectal cancer.

Peripheral blood was drawn from each subject and DNA was purified from peripheral-blood leukocytes.

Example 2: Genomic Sequencing

DNA was extracted from peripheral blood using the Nucleon DNA Extraction Kit, Scotlab, Lanarkshire, U.K. or using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN) as per the manufacturer's instructions. Each exon of hMSH2 and hMLH1 was amplified by PCR using 40 ng of genomic DNA in a volume of 50 μ L. Final reaction concentrations were 1 x PCR Buffer II (Perkin Elmer), 3.0 mM MgCl₂ (or 1.5 mM for hMSH2 exon 1), 0.2 mM dNTPs, 10 pmol of each specific oligonucleotide primer, and 1.25 units of Taq polymerase. Amplification was hot-started at 94°C for 3 minutes, followed by 35 cycles of 94°C for 20 seconds; 55°C for 20 seconds; 72°C for 40 seconds. The final reaction was extended at 72°C for 10 minutes, followed by storage at 4°C. Cycle sequencing used the PRISM Ready Dye Terminator Cycle Sequencing kit with AmpliTaq DNA polymerase, FS (Taq-FS; Perkin Elmer/Applied Biosystems) and an Applied Biosystems DNA Sequencer model 373A or 377 (Parker et al. BioTechniques 1996 21:694-699) according to the manufacturer's instructions. DNA sequence analysis was performed using Sequencher 3.0 (Gene Codes, Ann Arbor, MI) software by comparing published genomic sequences of hMLH1

(Han et al. Hum. Mol. Genet. 1995 4:237-242; Kolodner et al. Cancer Res. 1995 55:242-248) and hMSH2 (Kolodner, et al. Genomics 1994 24:516-526) with that of cancer cases or of random donors.

5 Examples of primers used for mutations in patients 815, 830 and 1157 are as follows:

(1) Patient 815, splice error in hMLH1 exon 1:

Forward primer:

5'-TGTAACGACGGCCAGTCTGAGGTGATTGGCTGAAG-3' (SEQ ID NO:
10 185)

Reverse primer:

5'-GGAAACAGCTATGACCATGCCGTTAAGTCGTAGCCCTT-3' (SEQ ID NO:
186)

(2) Patient 830, premature stop codon in hMSH2 exon
15 13:

Forward primer:

5'-TGTAACGACGGCCAGTCGATTAATCATCAGTGTAC-3' (SEQ ID NO:
187)

Reverse primer:

20 5'-GGAAACAGCTATGACCATGCAGAGACATACATTTCTATCTTC-3' (SEQ ID NO:
188)

(3) Patient 1157, missense in initial ATG of hMSH2
(exon 1):

Forward primer:

25 5'-TGTAACGACGGCCAGTCGCATTTTCTTCAACCAGGA-3' (SEQ ID NO:
189)

Reverse primer:

5'-GGAAACAGCTATGACCATGCCTCCCCAGCACGCGCC-3' (SEQ ID NO: 190)

**Example 2: In Vitro Synthesized-Protein-Truncation
30 Assay (IVSP)**

cDNA was generated by reverse transcription of RNA purified from lymphoblastoid cell lines from the affected

index case. PCR amplification of the cDNA was used to introduce a 17-bp consensus T7 promoter sequence and a mammalian translation-initiation sequence in frame with a unique hMLH1 or hMSH2 sequence. PCR primer sequences and conditions were similar to those previously described in Example 1. Each gene was amplified in two or three overlapping segments. Resultant PCR products were used in a coupled transcription-translation reaction (Promega) incorporating 2-5 μ Ci of 35 S-methionine. Labeled in vitro-transcribed protein products from the reaction were heat denatured and were analyzed by use of 8%, 10% and 12% SDS-PAGE gels. Gels were washed in fixative and were autoradiographed overnight at room temperature. All samples showing truncated protein products were reamplified independently, and an additional IVSP analysis was performed for conformation. For each analysis, normal control samples were run in parallel, and the wild-type full length protein was noted. In most analyses, artifactual bands were visible, presumably due to internally initiations since these were visible in samples from normal controls.

Example 3: Long Range PCR

For long range PCR of the novel mutation of hMSH2 discovered in patient 817, the GeneAmp XL PCR Kit (Perkin Elmer) was used with the following primers:

Forward primer:

5'-GGCCATTGTCACAGAGGATAAGA-3' (SEQ ID NO: 191)

Reverse primer:

5'-ACACAGCCCACGAAGGAGTG-3' (SEQ ID NO: 192)

The reaction mixture contained about 400 ng of genomic DNA in a volume of 50 μ L. Final reaction concentrations were 1 x PCR Buffer II (Perkin Elmer), 1.5 mM $\text{Mg}(\text{OAc})_2$, 0.8 mM dNTPs, 40 pmol of each specific oligonucleotide primer, and 4 units of rTth DNA polymerase. Amplification was hot-started at 94°C

for 1 minute, followed by 26 cycles of 94°C for 15 second and 68°C for 10 minutes. The final reaction was extended at 72°C for 10 minutes, followed by storage at 4°C.

Replicate cDNA sequencing of samples from patient 817 reproducibly demonstrated a truncation in hMSH2 due to deletion of the entire exon 13. However, extensive genomic sequencing failed to identify the mutation at the DNA level. Hence, the intronic region around exon 13 was analyzed by long range PCR to determine whether any large genomic deletion had completely removed that exon. Forward primer was in exon 12 and reverse in exon 14, giving around 15.5 kb wild type product. Using this approach, patient 817 was shown to carry a large deletion of approximately 3 kb which resulted in removal of exon 13.

15 **Example 4: Characterization of mutation in patient 579**

Characterization of the mutation in patient 579 was more complex. replicate hMSH2 IVSPs for patient 579 detected a very short protein fragment, which could not be explained on the basis of the His→Tyr mutation at codon 639, identified by genomic sequencing. Accordingly, additional genomic sequencing needed to be performed which resulted in identification of the second mutation at the splice acceptor site of exon 14. Using restriction-site changes induced by each mutation, both variants were traced through the family and were shown to reside on the same allele. Extensive sequencing of the reverse transcription-PCR products revealed that this complex double mutation results in an in-frame deletion of exons 12-14, thus accounting for the very short IVSP fragment. A His→Tyr mutation at codon 639 which results in a surrogate splice donor site and a 92-bp frameshift deletion of nucleotides 1914-2006, generating a premature termination codon 17 bp downstream of the exon 13 splice acceptor site has been described previously by Leach et al. Cell 1993 75:1215-1225 and Liu et al. Cancer Res. 1994

54:4590-4594. However, the 92 bp splice mutation reported to be present in this mutation was not present in patient 579, thus confirming that the double mutation in patient 579 is distinct from that reported by Liu et al. Cancer Res. 1994
5 54:4590-4594.

All publications including, but not limited to, patents and patent applications, cited in this specification, are herein incorporated by reference as if each individual publication were specifically and individually indicated to
10 be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention, including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further
15 elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. Therefore, the examples provided herein are to be construed as merely illustrative and are not a limitation of the scope of the present invention in any way.
20 The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

What is claimed is:

1. A variant human MLH1 or MSH2 gene comprising hMLH1 mutant 1, hMLH1 mutant 2, hMSH2 mutant 1, hMSH2 mutant 2 or hMSH2 mutant 3.
- 5 2. A method of diagnosing hereditary non-polyposis colorectal cancer in a patient comprising:
 - (a) obtaining a DNA sample from a patient; and
 - (b) screening the DNA sample for the variant human MLH1 or MSH2 gene of claim 1, wherein the presence of the variant
10 gene is indicative of hereditary non-polyposis colorectal cancer.
3. A method for predicting susceptibility of a patient to developing hereditary non-polyposis colorectal cancer comprising:
 - 15 (a) obtaining a DNA sample from a patient; and
 - (b) screening the DNA sample for the variant human MLH1 or MSH2 gene of claim 1, wherein the presence of the variant gene is indicative of a susceptibility to hereditary non-polyposis colorectal cancer.
- 20 4. A method of identifying mutants in splice donor or acceptor sites of a human MLH1 gene comprising sequencing splice donor or acceptor sites of the human MLH1 gene with intronic primers for the human MLH1 gene and analyzing the sequences to identify any mutants.
- 25 5. An intronic primer for human MLH1.
6. A method of identifying mutants in splice donor or acceptor sites of a human MSH2 gene, comprising sequencing splice donor or acceptor sites of the human MSH2 gene with intronic primers for the human MSH2 gene and analyzing the
30 sequences to identify any mutants.

7. An intronic primer for human MSH2.

8. A transgenic model system for colorectal cancer comprising cells expressing the variant human MLH1 or MSH2 gene of claim 1.

ABSTRACT

Variant human MLH1 and MSH2 genes are provided. Methods of using these variant genes to diagnose hereditary non-polyposis colorectal cancer (HNPCC) and/or determine a patient's susceptibility to developing HNPCC are also provided. Methods and compositions for identifying new variant MLH1 or MSH2 genes are also provided. In addition, experimental models for hereditary non-polyposis colorectal cancer comprising these variant genes are provided.

Docket No.
DEX-0054

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**NOVEL MUTATIONS IN HUMAN MLH1 AND HUMAN MSH2 GENES USEFUL IN DIAGNOSING
COLORECTAL CANCER**

the specification of which

(check one)

☒ is attached hereto.

☐ was filed on _____ as United States Application No. or PCT International
Application Number _____
and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

(Number)

(Country)

(Day/Month/Year Filed)

☐

(Number)

(Country)

(Day/Month/Year Filed)

☐

(Number)

(Country)

(Day/Month/Year Filed)

☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

<u>60/105,180</u>	<u>October 22, 1998</u>
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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Fourth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

Full name of fifth inventor, if any	
Fifth inventor's signature	Date
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Post Office Address	

Full name of sixth inventor, if any	
Sixth inventor's signature	Date
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SEQUENCE LISTING

<110> Robbins, David
Lin-Goerke, Juili L.
Ling, Jessica

<120> Novel Mutations in Human MLH1 and MSH2 Genes Useful in
Diagnosing Colorectal Cancer

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atcttcttct ggttcgtcag tatagagttg aagtttataa gaatagagct ggaaataagg 120
catccaagga gaatgattgg tatttggcat ataag 155

<210> 108
<211> 33
<212> DNA

<213> Homo sapiens

<400> 108

gtaattatct tccttttttaa tttacttatt ttt

33

<210> 109

<211> 25

<212> DNA

<213> Homo sapiens

<400> 109

ttaagagtag aaaaataaaa atgtg

25

<210> 110

<211> 32

<212> DNA

<213> Homo sapiens

<400> 110

attaataagg ttcatagagt ttggattttt cc

32

<210> 111

<211> 26

<212> DNA

<213> Homo sapiens

<400> 111

ttataaaaatt ttaaagtatg ttcaag

26

<210> 112

<211> 36

<212> DNA

<213> Homo sapiens

<400> 112

agtttggttaa attttttaaaa ttttattttt acttag

36

<210> 113

<211> 279

<212> DNA

<213> Homo sapiens

<400> 113

gcttctcctg gcaatctctc tcagtttgaa gacattctct ttggtaacaa tgatatgtca 60
gcttccattg gtgttggtggg tgttaaaatg tccgcagttg atggccagag acagggttga 120
gttgggtatg tggattccat acagaggaaa ctaggactgt gtgaattccc tgataatgat 180
cagttctcca atcttgaggc tctcctcatc cagattggac caaaggaatg tgttttacct 240
ggaggagaga ctgctggaga catggggaaa ctgagacag 279

<210> 114
<211> 23
<212> DNA
<213> Homo sapiens

<400> 114
gtaagcaaat tgagtctagt gat

23

<210> 115
<211> 25
<212> DNA
<213> Homo sapiens

<400> 115
agaggagatt ccaggcctag gaaag

25

<210> 116
<211> 21
<212> DNA
<213> Homo sapiens

<400> 116
tctttaattg acatgatact g

21

<210> 117
<211> 24
<212> DNA
<213> Homo sapiens

<400> 117
tttttgcttt tcttattcct tttc

24

<210> 118
<211> 33
<212> DNA
<213> Homo sapiens

<400> 118
tcatagtagt ttaaactatt tctttcaaaa tag

33

<210> 119
<211> 147
<212> DNA
<213> Homo sapiens

<400> 119
ataattcaaa gaggaggaat tctgatcaca gaaagaaaaa aagctgactt ttccacaaaa 60

gacatttatc aggacctcaa ccggttggtg aaaggcaaaa agggagagca gatgaatagt 120
gctgtattgc cagaaatgga gaatcag 147

<210> 120
<211> 85
<212> DNA
<213> Homo sapiens

<400> 120
gtacatggat tataaatgtg aattacaata tatataatgt aaatatgtaa tatataataa 60
ataatatgta aactatagtg acttt 85

<210> 121
<211> 20
<212> DNA
<213> Homo sapiens

<400> 121
ttagaaggat atttctgtca 20

<210> 122
<211> 10
<212> DNA
<213> Homo sapiens

<400> 122
actggcacca 10

<210> 123
<211> 24
<212> DNA
<213> Homo sapiens

<400> 123
gtggtataga aatcttcgat tttt 24

<210> 124
<211> 16
<212> DNA
<213> Homo sapiens

<400> 124
aaattcttaa ttttag 16

<210> 125
<211> 150
<212> DNA
<213> Homo sapiens

<400> 125
 gttgcagttt catcactgtc tgcggtaatc aagtttttag aactccttacc agatgattcc 60
 aactttggac agtttgaact gactactttt gacttcagcc agtatatgaa attggatatt 120
 gcagcagtca gagcccttaa ccttttttcag 150

<210> 126
 <211> 23
 <212> DNA
 <213> Homo sapiens

<400> 126
 gtaaaaaaaaa aaaaaaaaaaaa aaa 23

<210> 127
 <211> 22
 <212> DNA
 <213> Homo sapiens

<400> 127
 aaaagggtta aaaatgttga tt 22

<210> 128
 <211> 13
 <212> DNA
 <213> Homo sapiens

<400> 128
 ttaaaaaaatg ttt 13

<210> 129
 <211> 23
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<400> 129
 cattgacata tactgaagaa gct 23

<210> 130
 <211> 27
 <212> DNA
 <213> Homo sapiens

<400> 130
 tataaaggag ctaaaatatt tggaaat 27

<210> 131
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<212> DNA
<213> Homo sapiens

<400> 131
attatacttg gattagataa ctagctttaa atgggtgtat ttt

43

<210> 132
<211> 22
<212> DNA
<213> Homo sapiens

<400> 132
ttcactaatg agcttgccat tc

22

<210> 133
<211> 26
<212> DNA
<213> Homo sapiens

<400> 133
tttctatttt attttttggt tactag

26

<210> 134
<211> 134
<212> DNA
<213> Homo sapiens

<400> 134
ggttctgttg aagataccac tggctctcag tctctggctg ccttgctgaa taagtgtaaa 60
accctcaag gacaaagact tgtaaccag tggattaagc agcctctcat ggataagaac 120
agaatagagg agag 134

<210> 135
<211> 44
<212> DNA
<213> Homo sapiens

<400> 135
gtatgttatt agtttatact ttcgttagtt ttatgtaacc tgca

44

<210> 136
<211> 20
<212> DNA
<213> Homo sapiens

<400> 136
gttaccacaca tgattatacc

20

<210> 137
<211> 20
<212> DNA
<213> Homo sapiens

<400> 137
cttacgtgct tagttgataa 20

<210> 138
<211> 53
<212> DNA
<213> Homo sapiens

<400> 138
attttaattt tataactaaaa tattttacat taattcaagt taatttattt cag 53

<210> 139
<211> 200
<212> DNA
<213> Homo sapiens

<400> 139
attgaattta gtggaagctt ttgtagaaga tgcagaattg aggcagactt tacaagaaga 60
tttacttcgt cgattcccag atcttaaccg acttgccaag aagtttcaaa gacaagcagc 120
aaacttacaa gattgttacc gactctatca gggataaat caactaccta atgttatata 180
ggctctggaa aaacatgaag 200

<210> 140
<211> 24
<212> DNA
<213> Homo sapiens

<400> 140
gtaacaagtg attttgtttt ttg 24

<210> 141
<211> 24
<212> DNA
<213> Homo sapiens

<400> 141
ttttccttca actcatacaa tata 24

<210> 142
<211> 26
<212> DNA
<213> Homo sapiens

<400> 142
tttgtattct gtaaaatgag atcttt 26

<210> 143
<211> 30
<212> DNA
<213> Homo sapiens

<400> 143
ttatttgttt gttttactac tttcttttag 30

<210> 144
<211> 110
<212> DNA
<213> Homo sapiens

<400> 144
gaaaacacca gaaattattg ttggcagttt ttgtgactcc tcttactgat cttcgttctg 60
acttctccaa gtttcaggaa atgatagaaa caactttaga tatggatcag 110

<210> 145
<211> 26
<212> DNA
<213> Homo sapiens

<400> 145
gtatgcaata tacttttttaa tttaag 26

<210> 146
<211> 25
<212> DNA
<213> Homo sapiens

<400> 146
cagtagttat ttttaaaaag caaag 25

<210> 147
<211> 23
<212> DNA
<213> Homo sapiens

<400> 147
ctttacccat tatttatagg att 23

<210> 148
<211> 23
<212> DNA
<213> Homo sapiens

<400> 148
ttgtcacttt gttctgtttg cag

23

<210> 149
<211> 124
<212> DNA
<213> Homo sapiens

<400> 149
gtggaaaacc atgaattcct tgtaaacct tcatttgatc ctaatctcag tgaattaaga 60
gaaataatga atgacttgga aaagaagatg cagtcaacat taataagtgc agccagagat 120
cttg 124

<210> 150
<211> 20
<212> DNA
<213> Homo sapiens

<400> 150
gtaagaatgg gtcattggag

20

<210> 151
<211> 23
<212> DNA
<213> Homo sapiens

<400> 151
gttggataaa ttcttttgtc tat

23

<210> 152
<211> 25
<212> DNA
<213> Homo sapiens

<400> 152
tagtaggtat ttatggaata ctttt

25

<210> 153
<211> 22
<212> DNA
<213> Homo sapiens

<400> 153
tcttttcttc ttgtttatca ag

22

<210> 154
<211> 151

<212> DNA
<213> Homo sapiens

<400> 154
gcttggaccc tggcaaacag attaaactgg attccagtgc acagtttgga tattactttc 60
gtgtaacctg taaggaagaa aaagtccttc gtaacaataa aaactttagt actgtagata 120
tccagaagaa tgggtgttaa tttaccaaca g 151

<210> 155
<211> 36
<212> DNA
<213> Homo sapiens

<400> 155
gtttgtaagt cattattata tttttaaccc tttatt 36

<210> 156
<211> 21
<212> DNA
<213> Homo sapiens

<400> 156
aattccctaa atgctctaac a 21

<210> 157
<211> 21
<212> DNA
<213> Homo sapiens

<400> 157
cattgcttct agtacacatt t 21

<210> 158
<211> 36
<212> DNA
<213> Homo sapiens

<400> 158
taatattttt aataaaaactg ttatttcgat ttgcag 36

<210> 159
<211> 98
<212> DNA
<213> Homo sapiens

<400> 159
caaattgact tctttaaatg aagagtatac caaaaataaa acagaatatg aagaagccca 60
ggatgccatt gttaaagaaa ttgtcaatat ttcttcag 98

<210> 160
 <211> 19
 <212> DNA
 <213> Homo sapiens

<400> 160
 gtaaacttaa tagaactaa 19

<210> 161
 <211> 21
 <212> DNA
 <213> Homo sapiens

<400> 161
 taatgttctg aatgtcacct g 21

<210> 162
 <211> 21
 <212> DNA
 <213> Homo sapiens

<400> 162
 tcagtattcc tgtgtacatt t 21

<210> 163
 <211> 21
 <212> DNA
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<400> 163
 tctgttttta tttttataca g 21

<210> 164
 <211> 246
 <212> DNA
 <213> Homo sapiens

<400> 164
 gctatgtaga accaatgcag acactcaatg atgtgttagc tcagctagat gctgttgtca 60
 gctttgctca cgtgtcaa at ggagcacctg ttccatatgt acgaccagcc attttggaga 120
 aaggacaagg aagaattata ttaaaagcat ccaggcatgc ttgtgttgaa gttcaagatg 180
 aaattgcatt tattccta at gacgtatact ttgaaaaaga taaacagatg ttccacatca 240
 ttactg 246

<210> 165
 <211> 16
 <212> DNA

<213> Homo sapiens

<400> 165

gtaaaaaacc tggttt

16

<210> 166

<211> 19

<212> DNA

<213> Homo sapiens

<400> 166

ttgggctttg tgggggtaa

19

<210> 167

<211> 19

<212> DNA

<213> Homo sapiens

<400> 167

cgattaatca tcagtgtac

19

<210> 168

<211> 78

<212> DNA

<213> Homo sapiens

<400> 168

agtttaggac taacaatcca tttattagta gcagaaagaa gtttaaaatc ttgctttctg 60
atataatttg ttttgtag 78

<210> 169

<211> 205

<212> DNA

<213> Homo sapiens

<400> 169

gccccaatat gggaggtaaa tcaacatata ttcgacaaac tggggtgata gtactcatgg 60
cccaaattgg gtgttttgtg ccatgtgagt cagcagaagt gtccattgtg gactgcatct 120
tagcccgagt aggggctggg gacagtcaat tgaaaggagt ctccacgttc atggctgaaa 180
tgttggaaac tgcttctatc ctcag 205

<210> 170

<211> 23

<212> DNA

<213> Homo sapiens

<400> 170

gtaagtgcac ctccctagtcc ctt

23

<210> 171
<211> 23
<212> DNA
<213> Homo sapiens

<400> 171
gaagatagaa atgtatgtct ctg 23

<210> 172
<211> 21
<212> DNA
<213> Homo sapiens

<400> 172
ccacatttta tgtgatggga a 21

<210> 173
<211> 24
<212> DNA
<213> Homo sapiens

<400> 173
atttcatgta attatgtgct tcag 24

<210> 174
<211> 248
<212> DNA
<213> Homo sapiens

<400> 174
gtctgcaacc aaagattcat taataatcat agatgaattg ggaagaggaa cttctaccta 60
cgatggattt gggtttagcat gggctatatc agaatacatt gcaacaaaga ttggtgcttt 120
ttgcatgttt gcaaccatt ttcattgaact tactgccttg gccaatcaga taccaactgt 180
taataatcta catgtcacag cactcaccac tgaagagacc ttaactatgc ttatcaggt 240
gaagaaag 248

<210> 175
<211> 56
<212> DNA
<213> Homo sapiens

<400> 175
gtatgtacta ttggagtact ctaaattcag aacttggtta tgggaaactt actacc 56

<210> 176
<211> 19
<212> DNA

<213> Homo sapiens

<400> 176

cttctcatgc tgtcccctc

19

<210> 177

<211> 23

<212> DNA

<213> Homo sapiens

<400> 177

acgcttcccc aaatttctta tag

23

<210> 178

<211> 176

<212> DNA

<213> Homo sapiens

<400> 178

gtgtctgtga tcaaagtttt gggattcatg ttgcagagct tgctaatttc cctaagcatg 60
taatagagtg tgctaaacag aaagccctgg aacttgagga gtttcagtat attggagaat 120
cgcaaggata tgatatcatg gaaccagcag caaagaagtg ctatctggaa agagag 176

<210> 179

<211> 17

<212> DNA

<213> Homo sapiens

<400> 179

gtttgtcagt ttgtttt

17

<210> 180

<211> 21

<212> DNA

<213> Homo sapiens

<400> 180

catagtttaa cttagcttct c

21

<210> 181

<211> 21

<212> DNA

<213> Homo sapiens

<400> 181

attactcatg ggacattcac a

21

<210> 182

<211> 10
<212> DNA
<213> Homo sapiens

<400> 182
tgtgttttcag

10

<210> 183
<211> 171
<212> DNA
<213> Homo sapiens

<400> 183
caaggtgaaa aaattattca ggagttcctg tccaaggtga aacaaatgcc ctttactgaa 60
atgtcagaag aaaacatcac aataaagtta aaacagctaa aagctgaagt aatagcaaag 120
aataatagct ttgtaaatga aatcatttca cgaataaaag ttactacgtg a 171

<210> 184
<211> 20
<212> DNA
<213> Homo sapiens

<400> 184
atcccagtaa tggaatgaag

20

<210> 185
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 185
tgtaaaacga cggccagtct gaggtgattg gctgaag

37

<210> 186
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 186
ggaaacagct atgaccatgc cgttaagtcg tagccctt

38

<210> 187

<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 187
tgtaaaacga cggccagtcg attaatcatc agtgtac 37

<210> 188
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 188
ggaaacagct atgaccatgc agagacatac atttctatct tc 42

<210> 189
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 189
tgtaaaacga cggccagtcg cattttcttc aaccagga 38

<210> 190
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 190
ggaaacagct atgaccatgc ctccccagca cgcgcc 36

<210> 191
<211> 23
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 191

ggccattgtc acagaggata aga

23

<210> 192

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

<400> 192

acacagccca cgaaggagtg

20